THE CREATION OF IMMUNOLOGICAL TOLERANCE IN ADULT ANIMALS

V. Ya. Shevlyagin

Division of Immunology and Oncology (Head – Professor L. A. Zil'ber), N. F. Gamaleya Institute of Epidemiology and Microbiology (Director – Professor O. V. Baroyan) of the AMN SSSR
(Presented by Active Member AMN SSSR L. A. Zil'ber)
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The state of immunological tolerance is usually associated with the introduction of corpuscular or soluble antigens into animals in the embryonic period or during the first days of postnatal life. Clearly, the matter of greatest practical importance is the possibility of producing immunological tolerance artificially in adult animals. Some promising results in this field have been obtained by the creation of parabiosis between adult mice of closely related lines [3], and also after irradiation of animals with roentgen rays [1].

Schwartz and Dameshek [7] have shown that if the formation of antibodies to albumin is depressed in rabbits by means of 6-mercaptopurine, and these animals are then given an injection of albumin alone, they do not form antibodies to the albumin, but preserve at the same time the ability to form antibodies to other antigens. In this way it was shown to be possible to produce tolerance in adult rabbits in relation to human serum albumin.

We have tried to reproduce Schwartz and Dameshek's results obtained with human serum albumin, and to discover whether this method could be used to produce a state of immunological tolerance in adult rabbits to other antigens and, in particular, to human erythrocytes of blood group IV. By using these two antigens (albumin and erythrocytes) we may compare the method of producing tolerance by injecting antigens into adult animals, and the method of treating animals during the embryonic or immediate postnatal period. By means of the ordinary "classical" injection of antigens it was easy to obtain rabbits tolerant to human serum albumin [2], but it was impossible to suppress completely in rabbits the formation of hemagglutinins against human erythrocytes [5].

To study whether we could obtain monospecific cytotoxic sera, we also conducted experiments to produce in adult animals a state of immunological tolerance to the acid protein fraction prepared from the organs of mongrel albino rats.

EXPERIMENTAL METHOD

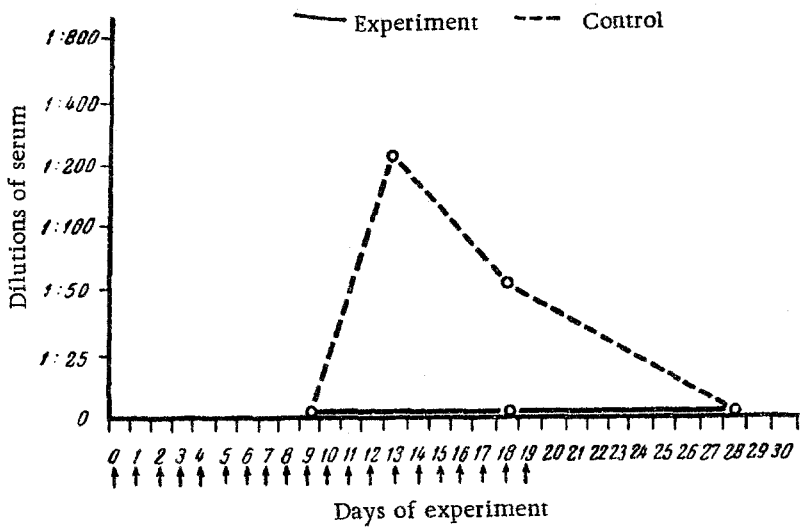
All the experiments were conducted on chinchilla rabbits (grey) weighing 2.0-2.5 kg. 6-Mercaptopurine (6MP) was dissolved in a 1 N solution of NaOH and the pH of the solution was adjusted to 7.8-8.0. The antimetabolite was injected intramuscularly into the rabbits strictly according to their weight.

The human serum albumin and the horse serum globulin were dissolved in physiological saline and preserved with merthiolate. Human group IV erythrocytes were obtained and kept in Olsver's solution at 4-6°. When repeated injections were given to a particular rabbit, erythrocytes obtained from the same donor were always used.

The acid protein fraction was prepared from a mixture of rats' organs (equal parts by weight of spleen, kidney, liver, lung, and stomach) and separately from rats' liver. The method of preparation of this fraction is well known: extraction of the tissue homogenate with weak alkali followed by removal of the precipitate obtained at pH 6.0 and dissolving the precipitate obtained at pH 4.5.

EXPERIMENTAL RESULTS

Experiments with Albumin. The group of experimental animals included three rabbits and the control group two rabbits. All the experimental rabbits received daily injections of 6MP intramuscularly, in a dose of 6 mg/kg body weight, for a period of 20 days. The first day of the experiment, i.e., the day on which the rabbits first received the antimetabolite, was regarded as the zero day, and the subsequent days were referred to as the 1st, 2nd, etc., days of the experiment.



Titers of antibodies to human serum albumin in the blood sera of experimental and control rabbits. The arrows denote injection of 6MP.

The results of Stavitsky's passive hemagglutination reaction [8], carried out on the sera of these animals, are shown in the figure, from which it is clear that 6MP totally suppressed the formation of antibodies in the experimental animals in response to the injection of albumin.

Two months later, when the rabbits had attained their original weight, the same animals received injections of human serum albumin or, as controls, of horse serum globulin. Tests on the sera from these animals showed that antibodies to human serum albumin were not formed in one of the experimental rabbits, but they were formed in the other experimental rabbits, although in much lower titers than in healthy animals. Well-defined antibody titers were observed in all three rabbits after injection of horse serum globulin.

The results of tests on the sera 4 months after administration of 6 MP to the animals showed that all the animals, including the rabbit in which antibodies had previously not been found, reacted clearly to injection of human serum albumin and also of horse serum globulin. These results suggest that tolerance to human serum albumin can be created in adult rabbits. In the rabbits in our experiments this state lasted not more than 4 months.

Experiments with Erythrocytes. We attempted to suppress the formation of antibodies in rabbits in response to the injection of human erythrocytes of blood group IV, and to discover whether these rabbits were tolerant to a subsequent injection of the same antigen. Sera from all the experimental and control rabbits were tested for the presence of natural hemagglutinins to human erythrocytes of blood group IV, starting with a dilution of 1 : 10. No hemagglutinins were found in the control and experimental rabbits.

Two groups of rabbits were used in the experiment. The first group consisted of 3 experimental and 2 control animals. The experimental animals received 6MP in a dose of 6 mg/kg daily for 21 days. One of the experimental rabbits died on the 23rd day. All the rabbits were given three intravenous injections of a 50% suspension of human erythrocytes of blood group IV.

Antibodies were formed in the experimental rabbits in response to the injection of human erythrocytes of blood group IV, but their titer was lower than in the control animals. In the course of the administration of 6MP to the rabbits, their titers of antierythrocyte antibodies fell, to rise again after the administration of 6MP had ended.

It may be supposed that the maximal suppression of the cells taking part in antibody formation does not take place at once, but during the course of administration of the antimetabolite, as it accumulated in the organism. The animals of the second group therefore received larger doses of 6MP (8 mg/kg for the first 6 days and 6 mg/kg for the next 12 days). The total dose of erythrocytes injected into each experimental rabbit was approximately the same – 6.75 ml in the first group and 6 ml in the second group.

The second group consisted of 4 experimental and 2 control rabbits. Two of the experimental rabbits died on the 8th and 15th day of the experiment. We were able to suppress antibody formation completely in one experimental

rabbit after giving it an injection of 6 ml of a 50% suspension of human erythrocytes of blood group IV. Hemagglutinins were found in the other experimental rabbit, but in much lower titers than in the controls. As soon as the first rabbit had reached its original weight, i.e., after approximately 2 months, it was given a second injection of 1 ml of a 50% suspension of erythrocytes from the same donor. The results of the investigation of the serum showed that in response to the injection of erythrocytes hemagglutinins were formed in the rabbit. Hence, in our experimental conditions, we were unable to obtain rabbits tolerant to human erythrocytes of blood group IV, notwithstanding the complete suppression of hemagglutinins after the first injection of erythrocytes in conjunction with 6MP.

Experiments with the Acid Protein Fraction Obtained from Rats' Organs. These investigations were carried out on two groups of rabbits, the first of which consisted of 4 experimental and 2 control rabbits, which received an antigen obtained from a mixture of rats' organs. The antigen was injected on the 3rd, 5th, and 7th days of the experiment, each rabbit receiving approximately 340 mg of protein altogether. The experimental rabbits also received injections of 6MP in a dose of 8 mg/kg during the first 6 days, and 6 mg/kg during the subsequent 12 days. Three of the experimental rabbits died on the 8th, 10th, and 18th days of the experiment.

The second group also consisted of 4 experimental and 2 control rabbits, which received antigen prepared from rats' liver in a dose of 400 mg protein per rabbit. The scheme of injection of the antigen and 6MP was the same as in the first group. In this group also, 3 of the 4 experimental rabbits died, on the 13th, 16th, and 17th days of the experiment.

Neither the ring precipitation test nor the precipitation in agar test, when performed on the sera of both groups of rabbits, demonstrated the presence of antibodies in the sera of the experimental animals, whereas they were present in the sera of the control animals.

Two months later, the control and experimental rabbits of both groups received three injections of the same antigens: antigen from the mixture of rats' organs in a total dose of 252 mg per rabbit of the first group, and antigen from rats' liver in a total dose of 275 mg per rabbit of the second group. The ring precipitation test revealed antibodies to the tissue antigens of the rats in the sera of the experimental animals, although in lower titers than in the controls. When these sera were investigated by the precipitation reaction in agar, the sera of the experimental animals gave fewer precipitation bands than the sera of the control rabbits. The most likely explanation of this result is that the experimental rabbits had become tolerant to certain tissue antigens and produced antibodies to others.

We were thus able to produce tolerance in adult rabbits to serum albumin, but not to human erythrocytes of blood group IV or to the soluble proteins of rats' organs. These results, which suggest that the method of inducing immunological tolerance by injecting adult animals simultaneously with antigen and 6MP is relatively effective, are in apparent agreement with the findings of other workers who have attempted to perform homotransplantation of skin in rabbits by simultaneously injecting the recipient with this antimetabolite. Meeker and co-workers [4], for instance, found merely that the duration of survival of the skin homografts was prolonged in the rabbits treated with 6MP. In no case were they able to obtain complete taking of the skin homograft. Similar results were reported by Schwartz and Dameshek [6].

It may be supposed that the efficacy of the immunological tolerance created in adult animals corresponds approximately to the efficacy of the tolerance created by injection of antigens into embryos or into animals during the first days of postnatal life. Tolerance to serum albumin can be obtained comparatively easily in rabbits by either method; yet neither method can produce tolerance to erythrocytes of a phylogenetically distant species. It is, nevertheless, highly probable that the combined use of both methods, together with the repeated injection of antigen along with 6MP into animals, will lead toward the solution of the problem of the creation of tolerance toward corpuscular and soluble antigens of distant species.

SUMMARY

Experiments were staged for the evolvement of immunological tolerance in adult rabbits with the aid of 6-mercaptopurine. After the administration of human serum albumin together with antimetabolite to rabbits, the animals formed no antibodies in response to subsequent albumin injection.

This stage of tolerance continued for a period of not more than 4 months. In analogous experiments with human erythrocytes of the group IV blood group and with soluble antigens, isolated from the rat organs, no state of immunological tolerance was achieved, notwithstanding complete depression of antibody formation during primary administration of these antigens together with 6-mercaptopurine. In repeated administration of soluble antigens of rat organs, the antibodies evidently developed only to some of these antigens.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
